Hepatotoxicity of Pyrrolizidine Alkaloids

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ABSTRACT - **PURPOSE:** This article aimed 1) to review herbal medicine containing pyrrolizidine alkaloids (PA)-induced toxicities of the liver; 2) to encourage the recognition and prevention of common problems encountered when using complementary and alternative medicine and 3) to review the toxic effects of herbal remedies containing PAs. **DESIGN AND METHODS:** We performed a systematic literature search using the PubMed and Google Scholar engines. The search was not restricted to languages. We also provide an interpretation of the data. **CONCLUSIONS:** Herbal remedies containing PAs can induce liver damage, including hepato- sinusoidal obstruction syndrome or veno-occlusive disease. Preventing overdose and monitoring long-term use of such remedies may avoid glutathione depletion leading to mitochondrial injury, and therefore avoid liver cell damage. Moreover, immediately stopping the herbal medication prevents further harm to the liver. Chronic consumption of hepatotoxicants can lead to cancer formation and promotion. The role of active metabolites in PA-induced liver toxicity and their mechanism of action require further investigation.

INTRODUCTION

Therapies consisting of complementary and alternative medicine (CAM) span a diverse group of substances that include herbal and dietary supplements (1). The use of CAM therapies increased dramatically in the last 20 years. MacLennan et al. (2) described the escalating use of CAM in Australia. In the United States, 65% of the population reported the use of CAM therapies (3-5). The out-of-pocket cost of purchasing non-vitamin, non-mineral natural products amounted to $14.8 billion in 2007 in the United States (6).
Herbal supplements can be associated with toxicities (7, 8). The frequency of hepatotoxicity attributable to plant-derived products is under-reported, since not all patients inform their clinicians that they use such therapies (9). As a result, hepatotoxicity associated with herbal use may be missed (10-13).

Indirect hepatotoxicants, as defined by Zimmerman (14), are substances that cause hepatic injury by producing selective biochemical or physiologic lesions which disrupt metabolic pathways or processes essential for the maintenance of parenchymal cell integrity. This is in contrast to direct hepatotoxins, which are agents that produce cholestasis, interfering mainly with biliary secretion, and usually sparing the parenchyma. Indirect hepatotoxicants include agents that disturb or competitively inhibit essential metabolites, alkylate or arylate key molecules, alter membranes, or produce other selective metabolic blocks and physiologic lesions. *Senecio* extract, a natural hepatotoxin, appears to fit this category in the experimental setting (15, 16).

Traditional medicines composed of herbs often contain a complex mixture of both beneficial and toxic phytochemicals. These are directly prepared from raw materials with little to no separation procedures. Acute poisoning due to the use of traditional medicines is a serious problem in South Africa. The inappropriate use of medicinal herbs has resulted in numerous fatalities, especially in young children (7, 17-23).

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It is currently estimated that 80% of the South African population consults with traditional healers and uses some form of traditional herbal medicine, usually in combinations (24, 25).

This review deals with phytochemicals of the cytotoxic group that are of special interest as classical, experimental hepatotoxicants, or as agents of importance to clinical medicine. The natural toxins discussed in this paper are excellent models of indirect hepatotoxicity. They include a number of agents of practical importance to human and veterinary medicine such as the pyrrolizidine alkaloids (PA) (16). PAs are well-studied hepatotoxins that produce selective biochemical lesions.

In figure 1 a (*Senecio doronicum*) and figure 1 b (*Senecio jacobea*) we illustrate two of the species of *Senecio*. The experimental hepatocytoxicity induced by natural substances has been described by several authors (15, 26).

Figure 1a *Senecio doronicum*

Figure 1b *Senecio jacobea*

Pyrrolizidine alkaloids

PAs are a class of alkaloids based on the structure of pyrrolizidine which are present in distinct plant families that grow worldwide (15, 16, 27) (Table 1.). PAs and PA N-oxides have been identified in plant species throughout the world (3% of the world's flowering plants) (28). PA hepatotoxicity, which has been long recognized, is predominantly observed after exposure to the following plant families: Boraginaceae *(Heliotropium sp., Trichodesma sp., Symphytum sp.* [Comfrey]), Compositae (*Senecio sp*. [Bush Teas], *Eupatorium* sp), *Crotalaria* sp. (Leguminosae), Greater Celandine (*Chelidonium majus*), and Ariaceae (*Castilleja* sp.) (28-48). Kava-kava (*Piper methysticum*) contains kava pyridone alkaloid, pipermethystine, which is abundant in leaves and stem peelings. This alkaloid induces mitochondrial toxicity. In addition, kavalactones such as 7,8- dihydromethysticin and desmethoxyangonin, abundant in the roots of the plant, may lead to different organ toxicities (46).

While sporadic in Western countries, PA poisoning is nearly endemic in areas where traditional remedies are frequently used, such as China (40), Africa and India (49-52). The toxicity of *Senecio* plants and related species has been shown to cause disease in grazing domestic animals. A series of studies has been reviewed by McLean (53). The toxic principles (PAs) have been identified, their presence in some plants has been established, and the characteristics of the hepatic injury they produce has been defined (53). The hepatotoxic and carcinogenic species of plant pyrrolizidine alkaloids (e.g. echimidine and jacobine), are generated by hepatic CYP2B6 and CYP3A4. Figure 2a presents precursors of pyrrole metabolites The acute hepatic injury produced by PAs includes zone 3 hepatic necrosis, minor steatosis, and occlusive injury of the small branches of the efferent venules (10, 53, 54). Chronic expoxure leads to the development of very large cells ("megalocytes"), cirrhosis and hepatocellular carcinoma (53). The mechanism of injury appears to involve the injury of the hepatic vasculature and parenchyma by pyrrole derivatives that react with DNA (55-57).

All PAs contain the core structure of pyrrolizidine, which consists of two fused penta-rings with a nitrogen atom at position four, one of the two points of fusion. The hepatotoxic derivatives are esters of 1-hydroxymethyl-1,2-dehydro-7-hydroxy-pyrrolizidine. Requirements for toxicity appear to include the 1-2 unsaturation and the esterification

with a branched-chain acid (53). These 1-2-unsaturated PAs are metabolized by CYP450 enzymes in the liver to the corresponding "pyrrole type esters", responsible for PA-induced toxicity. Saturation of the double bond eliminates the toxicity; esterification does not play a major role in toxicity. Solubility of the alkaloids in aqueous solvents is low at a neutral pH, and is enhanced by acidification (53, 58). Pyrrolizidine alkaloids consist of a necine base and necic acid. The necic acids are four to six carbon-containing mono- or di-carboxylic acids. The structures of the four representative types are necine bases, platynecine, retronecine, heliotridine, and otonecine. The platynecine type PAs, which do not contain a double bond in the necine base, are considered weakly toxic. However, the retronecine-, heliotridine-, and otonecine-type that have a double-bond at the C1 and C2 positions of the necine base exhibit high levels of hepatocytotoxicity (10).

Laboratory measurements

Both acute and chronic PA-induced toxicity have been reported in humans depending on the quantity and frequency of the exposure (59). Urine and blood samples belonging to patients who have been exposed to traditional medicine can be used to identify the quantity of PA that is present, which can help monitor chronic or acute PA toxicity. Steenkamp et al. (60) and Neuman and Steenkamp (51) showed an increased risk of veno-occlusive disease in people who used herbal remedies containing PAs, despite no previous medical history. In addition, no herb-therapeutic or herb-herb interaction was noted, since the patients did not take any barbituates, benzodiazepines, tricylic antidepressants, paracetamol, or other herbal remedies. Moreover, there are several reports of using the herbal material to livestock. *Senecio oxyriifolius DC* is given to animals with swelling and *Senecio tamoides DC* is administered to animals with anthrax (61).

Analytical chemistry techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectroscopy (MS), and thin layer chromatography (TLC), in tandem with each other, have been conducted by past researchers to provide chemical identification and quantitative data regarding the amount of PAs present in blood and urine (22, 62-68). Although analytical techniques such as liquid chromatography (LC) and GC are currently available to detect traces of PAs, there are no rapid screening assays to assess levels of PAs on a commercial scale to be adopted in the food regulation industry. However, commercial scale analytical methods are being explored as a way to sensitively detect toxic PAs from complex mixtures. The lack of reference standards for analysis is a great hindrance for screening methods to be implemented (69). Oplatowska et al. are in the process of developing a rapid enzyme-linked immunosorbent assay (ELISA) capable of performing a multiplex immunoassay for PAs with N-oxide (70). Griffin et al. are also investigating a LC-ion trap MS method on a commercial scale to detect PAs in honey (71), whereas Martinello et al. are finding quantitative methods to measure PA content by UPLC-MS to extrapolate on an industrial level (72).

Pyrrolizidine alkaloids toxicity

PAs are found in more than 6,000 plants within the *Asteraceae*, *Boraginaceae*, *Compositae*, and *Fabaceae* families (28, 73-75). In total, over 300 different PAs have been identified, and they are categorized according to their necine base and necic acid components (76, 77). Plants that are part of the *Senecio* genus are reported to have high amounts of PAs in the form of senecionine, retrorsine, riddeliine, integerrimine, neosenkirkine, and florosenine (27, 78). Not all PAs are toxic. However, the ones that are reported to be toxic all commonly have a double bond in the ring nucleus, an esterified hydroxyl group, and a branched carbon in at least one of the ester side chains (76, 77, 79). Senecionine, retrorsine, and riddelliine are three of many PAs that meet these criteria. Despite their high content in toxic PAs, plants of the *Senecio spp.* are commonly used in traditional medicine for numerous purposes. PA-induced toxicity manifests its problematic nature in two dimensions: firstly, grazing livestock experience irreversible and fatal liver damage by feeding off plants that are rich in PA or feeds that are contaminated with plants rich in PA; and secondly, individuals treated with traditional medicine or exposed to contaminated food, including both contaminated plants and products from infected animals, may experience toxicity.

Senecio-induced liver injury is a commonly reported issue for livestock. Countries such as Zimbabwe (80), South Africa (81), the United Kingdom (82), Belgium (83), France (84), Australia (85), Spain (86), Brazil (87-91), Canada (92), and the United States (93, 94) have all reported PA-

induced livestock deaths after post-mortem investigation. *Senecio*-induced liver poisoning in animals is preventable, and represents a high cost for farmers. Approximately 10% of cattle and 5% of small livestock are poisoned by PA-rich plants annually in South Africa, which is equivalent to approximately 8,107,521 South African rand in financial cost per year (81). Stuart et al. (95) reported Cocklerbur (*Xanthium strumarium*) intoxication in swine, while Witte et al. (96) describe the same phenomenon in cattle. Ninety per cent of the complaints received by the United Kingdom Department for Environment, Fisheries and Rural Affairs are from farmers regarding weed catastrophes such as *Senecio* (97). The Australian dairy industry loses $2,428,211 per year in milk production and $434,327 per year in beef production because of *Senecio jacobaea*. Overall, more than $4.0 million is lost in the Australian agricultural industry solely due *to Senecio jacobaea* (98). The United States Department of Agriculture have reported that cattle, deer, horses, and goats are exposed to plant toxins by accidental exposure or dried as part of silage or hay (99, 100). It thus provided suggested techniques to help mitigate the problem such as crop rotation techniques, proper forage harvesting, and control of weed invasion by pulling weeds mechanically or using broadleaf herbicide to mitigate the harm of PA-rich plants on livestock viability (100). Nevertheless, both developed and developing countries that face PA-induced livestock death lack detailed agricultural protocols that would assist farmers from livestock attrition. Basic science research must be communicated coherently and detailed protocols must be implemented for farmers and land managers in order to improve agricultural conditions.

Medicinal tea containing PA-rich herbs also induced serious liver veno-occlusive disease in many parts of the world, with numerous people ending up with severe liver damage and death (31, 34, 101-103). Although analytical methods have confirmed that numerous herbal teas contain PAs, which is detrimental to health, there are still licensed and registered commercial products that are available in the market for consumers to purchase (104). For example, comfrey has been removed from the market in France following numerous reports of liver damage. It remains widely available in the United States despite the US Food and Drug Administration's requests to remove comfrey products from the market (105).

Special populations are more vulnerable to products containing PAs. Many of PA containing herbal teas are marketed for pregnant or lactating mothers; traces of PA may be exposed to infants, harming the mother's child (106). Another source of exposure to PA is from food contamination. Honey from nectar of PA-rich flowers is often contaminated with PAs according to LC-MS results, and the presence of PAs in honey is not uncommon (107-113). Milk could also contain traces of PAs if the cows eat *Senecio*-contaminated feed (114). An outbreak of hepatic veno-occlusive disease (VOD) in Western Afghanistan occurred after people were exposed to PA-contaminated wheat flour (115).

A cohort study on Ugandan participants showed an increased risk of liver fibrosis when an individual uses traditional medicine composed of herbs from the *Asteraceae*, *Fabaceae*, and *Lamiaceae* families (116). This is problematic since many Ugandan patients with human immunodeficiency virus resort to traditional medicine and neglect their antiretroviral therapy, which may potentiate non-compliance to their prescribed treatment and by itself, or in combination with alcohol, may lead to hepatotoxicity (52, 117).

Zimmerman (10) emphasized that the rules for "causality assessment" in attributing liver injury to any toxin are similar to those for other therapeutics (e.g. exposure must lead to the onset of liver injury and other liver diseases should be excluded). The severity of liver injury is enhanced by repeated exposure. The mainstay of therapy for herbal hepatotoxicity is withdrawal of the offending toxin. Liver injury may improve after stopping the ingestion of the toxin. However, in fulminant cases, an abrupt decline in liver biochemical tests may indicate worsening of the liver status rather than improvement of its functional capacity. As a consequence, early recognition of toxicity is important to permit assessment of severity and monitoring for acute liver failure (118). More extensive disease can lead to cirrhosis, hepatic failure, and death (14). The acute form is rapidly fatal in 20 to 40 percent of patients (with worse prognosis in adults compared with children). Approximately 15% of patients with acute disease will progress to subacute or chronic injury, succumbing within several years to end stage liver disease. Some of the patients develop cirrhosis and portal hypertension (14).

Regardless of the intended use of traditional

remedy, clinical case reports have been published regarding PA-induced hepatotoxicity; thus, the toxicology behind *Senecio* spp. and other PA-rich herbs should not be neglected or undermined. Moreover, monitoring the levels of PA in blood and/or urine will help in diagnosing and treating the toxic reaction.

Hepatic sinusoidal obstruction syndrome

Numerous case reports in human and veterinary medicine indicate that PAs at high doses induce sinusoidal obstruction syndrome (HSOS) previously referred to as hepatic veno-occlusive disease (VOD). Without medical intervention and discontinuation of the toxicant exposure, HSOS can lead to more severe complications such as liver fibrosis, cirrhosis, necrosis, and ultimately death (119). Willmot and Robertson first clinically described the symptoms of HSOS by ascites, hyperbilirubinemia, hepatomegaly, and abdominal pain (119-121). Non-thrombotic luminal occlusion of the small centrilobular veins leads to hepatic congestion and subsequent hemorrhagic parenchymal necrosis (10). More extensive disease can lead to hepatic failure, and death (10). Regardless of the cause, HSOS begins with injury to the hepatic venous endothelium. The preexisting liver disease increases the risk of developing HSOS. Preexisting liver disease may impair metabolism of drugs and phytochemicals, and thus predispose to cell injury. In addition, patients with chronic viral or alcoholic hepatitis may have abnormalities in hepatic endothelial cells that could make them more susceptible to PAs (15). Endothelial cells in patients with hepatitis may abnormally express adhesion molecules and procoagulant factors. The acute form is rapidly fatal in 20 to 40 percent of patients (with worse prognosis in adults compared with children). Approximately 15 percent with acute disease will progress to subacute or chronic injury, succumbing within several years to end stage liver disease (10). Early pathologic changes include the deposition of fibrinogen and factor VIII within the venular walls and liver sinusoids (10).

HSOS is also a serious complication that is not uncommon for hematopoietic stem cell transplant patients and exhibit similar pathological features (121). The pathogenesis of HSOS is elucidated employing *in vivo* and *in vitro* studies to understand its molecular basis. Studies suggest that endothelial cell injury via oxidative stress and PA-induced apoptosis of hepatocytes is one contributing factor. *In vivo* and *in vitro* models show concentration-dependent PA-induced depletion of glutathione indicative of oxidative stress by PAs (122). One proposed mechanism implicates PAs in inhibiting hepatocyte proliferation and inducing cell death is by decreasing the level of Bcl-x, an anti-apoptotic protein, and increasing the level of Bax, a pro-apoptotic protein that enhances the release of cytochrome c from the mitochondria for apoptosis (123). Another proposed mechanism to explain PA-induced apoptosis involves reduced p53expression, which is independent from Bcl expression (124). Zuckerman et al. have conducted an *in vitro* study and demonstrated that toxic PAs not only induce apoptosis, but also clump the tubulin cytoskeleton at relatively low concentration, and lead to necrotic cell bodies (125). The inflammatory response is also suggested to be part of the pathogenesis of PA-induced HSOS (16). Cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and endothelin-I (ET-1) are secreted by monocytes in response to PAs (50, 126). TNF-α is a cytokine that directly induces cell death in normal endothelial cells (127). The pro-coagulant properties of TNF-α and IL-1β and the coagulation pathway remain active areas of research in understanding HSOS (128, 129).

As a result of liver injury, bile acid homeostasis is also compromised. Xiong et al. have investigated PA-induced hepatotoxicity and its change in metabolomics and genomic profiles in hepatocytes (130). Genome microarray analysis and quantitative polymerase chain reaction (qPCR) were used to observe and verify the down-regulation of key enzymes associated with bile acid homeostasis such as CYP7A1, bile acid CoA-amino acid *N*-acetyltransferase, sodium taurocholate cotransporting polypeptide, and organic anion transporting polypeptide, and the up- regulation of multidrug-resistance-associated protein 3. Patient serum metabolomic analysis via ultraperformance liquid chromatography (UPLC) showed an elevated activity of alanine aminotransferase, aspartate aminotransferase, and increased concentration of total bilirubin, all indicative biomarkers to hyperbilirubinemia associated with HSOS. A murine model conducted by Xiong et al. showed similar results, with elevated level of alanine aminotransferase, aspartate aminotransferase, and total bilirubin concentration, all indicative biomarkers of hepatotoxicity (131).

PAs undergo three main metabolic pathways. PAs that are hydrolyzed to a carboxylic acid or N-

oxidized to a N-oxide metabolite are non-toxic and soluble in water and thus excreted *via* urine (53). Although PAs in their native form are non-toxic, when they are biotransformed by CYP3A their metabolite reacts with nucleic acids and proteins (132-135). The initial oxidation by CYP3A, then the spontaneous dehydrogenation of the necine ring produces a dehydro-pyrrolizidine compound, a toxic pyrrolic ester that acts as an electrophile. Thus, CYP3A inducers could increase the susceptibility of PA-induced toxicity, while CYP3A inhibitors could prevent toxic outcomes (136), since inhibitors yield less dehydropyrrolizidine alkaloids (137). Although pyrrolizidine N- oxide metabolites are generally non-reactive and get excreted via urine, excess of these metabolites can be further biotransformed into toxic epoxides and necine bases, which is detrimental to cellular function (138). At low concentrations, endogenous detoxification systems, notably glutathione, would conjugate the electrophilic metabolite, stabilizing it for excretion via urine. *In vivo* and *in vitro* studies have shown that PA-induced HSOS has been linked to the depletion of glutathione in sinusoidal endothelial cells, indicative of PA-induced oxidative stress (122). Cattle with irreversible liver damage induced by *Senecio* spp. showed higher activity of copper-zinc superoxide dismutase and thiobarbituric acid-reactive substances, and higher level of non-protein sulfhydryl group, all indicative of lipid peroxidation and oxidative stress as contributing pathological pathways (59). Glucuronidation of PA has yet to be extensively investigated but the study by He et al. showed that senecionine and other PAs are conjugated by glucuronic acid in humans and animals (139).

PA toxicity is dose- and time-dependent

HepG2 cells have shown senecionine-induced dose- and time-dependent cytotoxicity assessed by MTT, bromodeoxyuridine incorporation assay, neutral red uptake assay, resazurine assay, lactate dehydrogenase release assay, and sulforhodamine B assay (16, 140). Moreover, cell lines and injection bioassays show that PAs are cytotoxic in a dose-dependent manner (141). L-02 cells show in a dose- and time-dependent manner that senecionine and other PAs such as adonifoline, senecionine, monocortaline, and isoline deplete cellular glutathione levels and increase the level of oxidized glutathione, resulting in a decreased ratio of glutathione to oxidized glutathione (122, 141). N-acetyl-cysteine, the precursor to glutathione, an antioxidant compounds, lowered the susceptibility of PA-induced hepatotoxicity (122, 143), while a glutathione synthesis inhibitor increased the susceptibility to PA-induced hepatotoxicity (143). Primary mice hepatocytes have shown that senecionine and other PAs induce apoptotic DNA laddering, caspase-3 activation, and a decreased level of Bcl-xL, an anti-apoptotic protein (144), thus concluding that PAs share a common hepatotoxic signaling pathway that involves the degradation of Bcl-xL protein and activation of the intrinsic apoptotic pathway, mediated by the mitochondria.

Our previous research regarding *Senecio*-induced toxicity has showed that an aqueous extract of *Senecio* induced cytotoxicity in a dose-time-dependent manner, as determined by ELISA and terminal dUTP nick-end labeling in HepG2 cells. Furthermore, glutathione depletion was observed when cells were treated with *Senecio* extract and N-acetyl-cysteine was shown to potentially reduce cytotoxicity induced by *Senecio*. Lastly, caspase-3 and caspase-9 inhibitors were demonstrated to prevent apoptosis associated with aqueous *Senecio* extract (145). A proposed mechanism of toxicity is illustrated in Figure 2b.

In hepatocytes, CYP - P450s convert dehydro-pyrrolizidine alkaloides (PA)s to 6,7-dehydro-pyrrolizine esters which represent the toxic metabolites. Dehydro-retronecine and dehydro-heliotridine are produced from the initial toxic metabolites via reactive oxygen species. Esterases can detoxify dehydro-PAs by hydrolyzing their ester groups to produce non-toxic necic acids and necines. Dehydroretronecine and dehydroheliotridine react rapidly with nucleophiles such as SH, OH, NH groups on nucleotides, as well as with proteins to form adducts. Glutathione is responsible for detoxification of the toxic metabolites in the liver. Endothelial cells lining the sinusoids of the liver became damaged due to an accumulation of toxic metabolites. This phenomenon causes the obstruction of the sinusoids.

Over time, some tissue-bound adducts release dehydroretronecine/heliotridine which are less reactive toxic metabolites than the dehydroretronecine and dehydroheliotridine toxic metabolites. These metabolites form new adducts leading to chronic diseases such as cancer, pulmonary hypertension, fibrosis of the liver and cirrhosis.

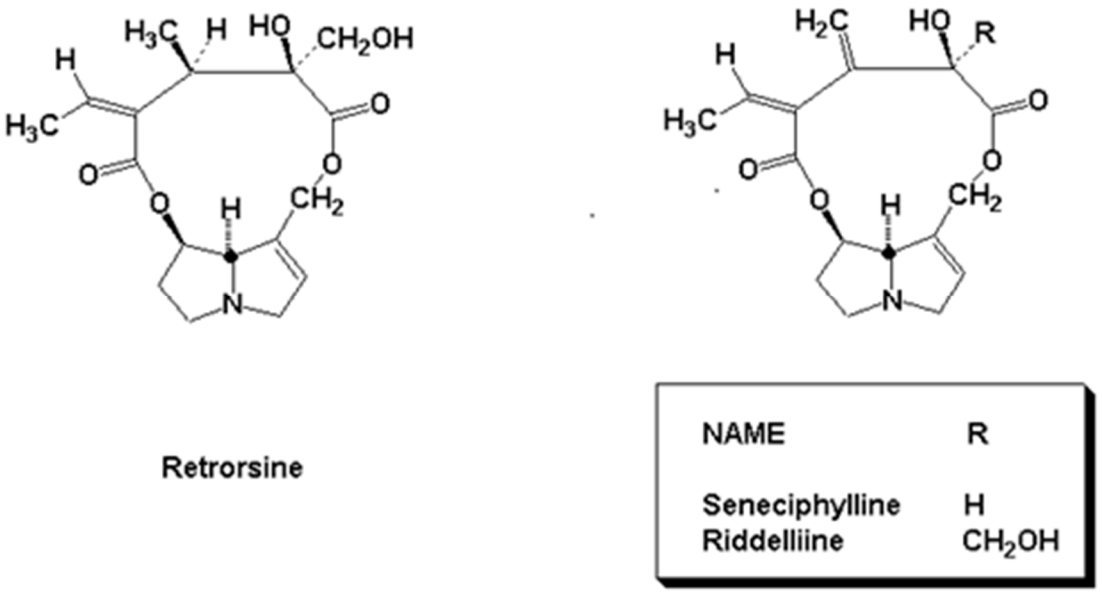

Figure 2a

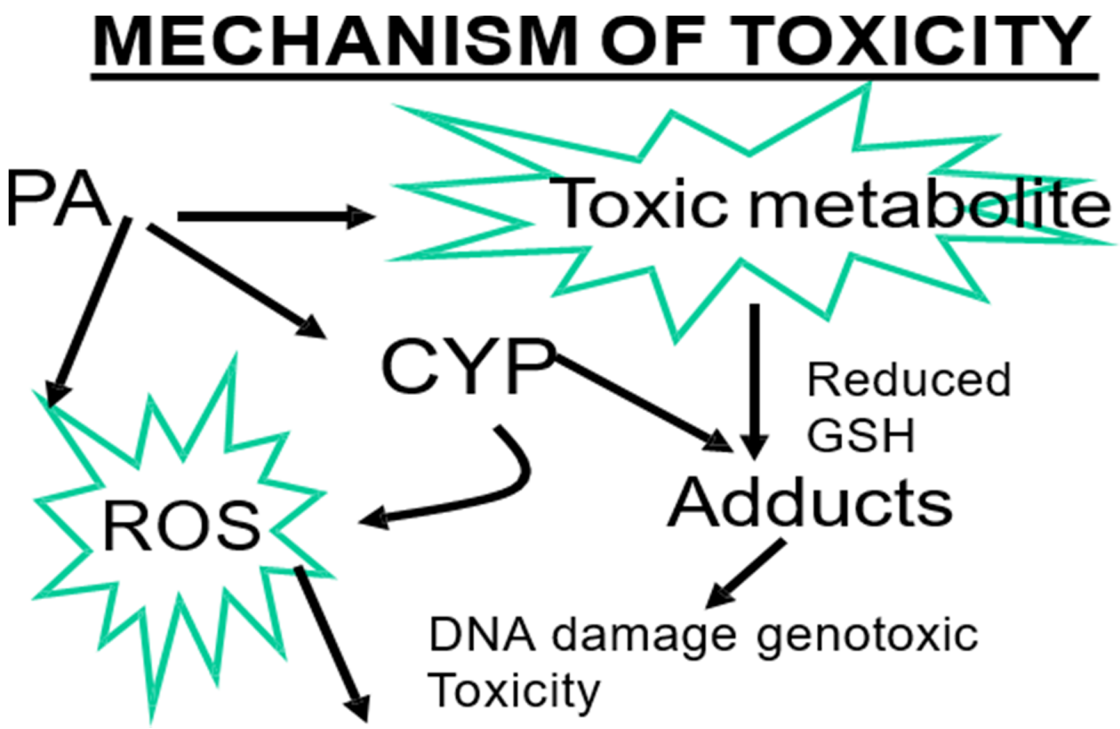

Figure 2b. CYP – Cytochrome P450s, PA-pyrrolizidine alkaloids, RSO - reactive oxygen species, GSH-Gluthatione.

PAs and cancer

Culvenor firstly reported the tumor-inhibiting

activity of PA isolated from *Senecio mikanioides* due to the compound's ability to alkylate biological molecules (146). Further investigation found indicine N-oxide as a lead compound for cancer therapy since less severe hepatotoxicity in animal models was shown relative to other PAs (147). Indicine N-oxide is a PA found in *Heliotropium indicium* and has been produced semi-synthetically for phase I and II clinical trials in patients with advanced solid tumors and leukemia (148-151). Kovach et al. noticed that administering daily doses of 3.0 g/m^2 for five consecutive days to advanced cancer patients showed symptoms of myelosuppression but no significant hepatic and renal impairment (152). Furthermore, three patients with advanced gastrointestinal cancer had no definite therapeutic response to indicine N- oxide and its ability to treat solid tumors (152).

The clinical trial of indicine N-oxide in acute leukemia patients conducted by Letendre et al. failed to show therapeutic improvement. Five out of eleven patients experienced severe hepatotoxicity and four resulted in death due to liver failure (153). A five year-old boy with acute myelocytic leukemia was given 7.5 g/m^2 indicine N-oxide after two complete remissions with standard chemotherapeutic agents. Three days later, the patient showed signs of acute hepatic failure, and died nine days later with hepatic necrosis (154). A phase I clinical trial was carried out by Whitehead et al. using indicine N-oxide to treat children with leukemia or solid tumors who failed to show progress on standard chemotherapy. Myelo-suppression and hepatotoxicity limited the dose of indicine N-oxide in pediatric patients with solid tumors and leukemia, respectively (150). Further, no clinically significant objective responses were noted in patients, thus disappointingly ceasing the scientific investigation into indicine N-oxide. Miser et al. (155) carried out a phase II clinical trial in children with relapsed acute leukemia and although indicine N-oxide showed some anti-leukemic activity, it was associated with severe and irreversible hepatotoxicity. PA appears to be a compound of interest for anti-cancer therapy due to its cytotoxic and angiogenesis inhibiting property. However, as a result of its severe hepatotoxicity, clinical application is limited.

PAs found in several commonly consumed plants are potential carcinogens or tumor promoters and should be avoided (156). Also, the Traditional Indian medicine, Ayurveda, uses medicinal plants which contain toxic PAs which represent a severe mutagenic and cancerogenic risk (157). The carcinogenic species of PAs (e.g. echimidine and jacobine), namely pyrrole-type metabolites, are generated by hepatic CYP2B6 and CYP3A4 (158). Roeder and his team described PA chemical structure and the use of PA-containing remedies in the world (159-161). In their recent review article, Roeder et al. (162) also mention that PAs are mutagenic, carcinogenic, and teratogenic. In addition, the authors recognized the need to identify and to regulate PA-containing medications. While the mainstay of therapy for herbal hepatotoxicity is withdrawal of the offending toxin, early recognition of toxicity is important to permit assessment of severity and monitoring for liver damage. However of importance is prevention of the toxic event.

Regulation of herbal remedies

Regulation of the safety, efficacy and quality of herbal remedies is greater in the Europe (EU) than in the United States (US). Complementary and Alternative Medicine in the United States receives recommendation from the Institute of Medicine Committee on the Use of Complementary and Alternative Medicine regarding the possible use of herbal remedies by the American Public (163). In addition, FDA regulates the safety and efficacy of the complementary and alternative medication as well as of the amount of PA in food and natural related products (164-169). EU requires manufacturers of all over-the-counter herbal products to register and license the product with the European Agency for the Evaluation of Medicinal Products. A premarket evaluation of quality and safety of the product is required. Companies have been asked to carry out post- marketing surveillance and report serious adverse events. Instead of requiring new rigorous efficacy studies to market a new product, documentation from the medical literature of safety for the relevant condition and reasonable plausibility of efficacy is needed. The World Health Organization (WHO) developed a Traditional Medicine Strategy to promote the safety of the complementary and alternative medicine as well as to regulate the poisonous substances of natural origin that may be found in food (164). As part of this effort, the WHO distributed a survey about national policies and regulations for herbal medicines to help frame regulatory policies, enhance quality, safety and efficacy. The potential PA contamination of

food, herbal medicine, herbal teas, dietary supplements or food containing PA plant material representing potential threat to human health by PAs is studied. In pharmaceuticals, the use of these plants is regulated.

Drying pollen granules during processing reduces the dehydroPA content of the granules (173). Nevertheless, 17 (31%) of 55 commercial bee-pollen products purchased at retail outlets in Europe have been found to contain 1080– 16350 mg/kg[1] of dehydro-PAs mainly from *Echium* species (172). *Echium sp*. pollen has a characteristic deep purple colour and bee-collected pollen granules from Echium *sp*. have been observed, and in some cases confirmed by scanning electron microscopy, while *Senecio* pollen cannot be distinguish from the other plant's pollen.

Consideration of the PA concentrations observed in honey from PA containing plants and pollen products led to an international regulation of PAs in food. Safe doses for PA in dietary and herbal remedies as well as in honey and pollen in different EU countries (171-174).

Harmonization of the market for herbal medicines is a fundamental requirement for European industries and health professionals. Herbal medicines can be also sold as food supplements, which required a common regulatory status in the various European countries. The European Parliament and by the Council of Europe established that herbal medicines released in the market need authorization by the national regulatory authorities of each European country and that these products must have a recognized level of safety and efficacy. In 1992, the Federal Health Department of Germany has restricted "the manufacture and use of pharmaceuticals containing pyrrolizidine alkaloids with a unsaturated necine skeleton". The herbal plants "may be sold and used only if daily external exposure to no more than 100 µg pyrrolizidine alkaloids and internal exposure to no more than 1 µg per day for no more than six weeks a year.

In Europe, marketing of traditional herbal medicinal products is regulated by an ad hoc Directive (i.e. Directive 2004/24/EC). Food Standards Australia New Zealand (FSANZ) has proposed a TDI for dehydro-PAs of 1 µg kg body weight/day. This is higher than those determined by other authorities partly due to FSANZ considering the cancer risk to humans to be unproven and also to be unlikely due to a more efficient DNA-repair system in humans (FSANZ 2001). FSANZ recommends dilution of contaminated product with uncontaminated product to achieve the recommended level (FSANZ 2004), a practice not allowed in some other jurisdictions (European Food Safety Authority (EFSA) 2007). In addition, Senecio spp. is prohibited for therapeutic use in Australia.

The safety of herbal medicinal products needed to be evaluated on the basis of existing scientific literature (data from clinical studies, case reports, pre-clinical studies). Based upon the safety and efficacy data, herbal medicinal products are categorized as: (i) medicinal herbs with a recognized level of safety and efficacy; and (ii) traditional used medicinal herbs that do not have a recognized level of efficacy but are acceptably safe (175-183).

DehydroPAs relative to consumption of honey containing dehydroPAs tolerable daily intakes was determined by German (Bundesanzeiger 1992) and Dutch (Rijksinstituut voor Volksgezondheid en Milieu (RIVM) 2007) to be 0.1 microgram/day. Australian and New Zealand (Food Standards Australia-New Zealand (FSANZ) 2001) authorities (175-183).

Not all the countries regulate the type as quantity of PA containing foods and remedies. Qianliguang is traditional herbal medicine growing in different locations of China. Significant diversity of the PA types and quantities were revealed among the samples tested (184-185). The estimated total amounts of toxic PAs in some of the samples exceed the toxic limits of PA intake restricted by WHO, demonstrating the timely and high demand for regulating both types and quantities of PAs present in Qianliguang as well as food containing PAs (184-185). Moreover, Qianliguang is used also as herbal remedy in Europe. The United Kingdom Medicines and Healthcare Product Regulatory Agency restricted PAs in this herbal product to 1 mg/day (or 16.7 µg/kg/day for a 60-kg body weight) for 2 weeks and to 0.1 µg/day (1.67 µg/kg/day) for a longer period (186). Pharmacopoeia of P.R. China (2010 edition) stated that adonifoline in Qianliguang should not exceed 0.004% (40 µg/g of dried herb) (187). However, in addition to adonifoline there are other PAs (retronecine and the toxic otonecine-type PAs)in the same herb (185).

CONCLUSION

PA-induced toxicity demonstrates the lack of pharmacovigilance regarding traditional medicine and herbal supplements. Therefore, a greater degree

of safety regulation is required to assess the toxicological profiles of traditional medicine and herbal supplements that are available to the public. Moreover, stronger agricultural protocols to mitigate *Senecio* poisoning in livestock, as well as regulatory policies regarding safety of traditional medicine, is of utmost importance. In addition, individuals with pre-existing liver injury or simultaneously taking drugs of use or misuse that induce certain cytochrome p450s may be more susceptible to PA-induced hepatotoxicity. Also should be taken into account the likelihood and significance of intermittent level dietary exposure to PA as well as the potentiating effects of environmental copper and genetic factors. Another important factor is that the methods of determining the quality and quantity of PAs in remedies and foods should be standardized.

The work presented in this paper may contribute to finding a rational approach to limit or prevent liver damage due to herbal remedies. Based on our clinical cases of herbal remedy-induced liver damage, this subject deserves further investigation. From a social point of view, the present investigation on the mechanism of herbal remedy-induced HSOS may assist in gaining a larger recognition of the problem, which will be required for the development of educational strategies aimed at informing physicians and the public about the potential dangers of these commonly used remedies. A recommended strategy is to inform the consumers in articles which are written in plain language, as well as, answering the key questions an individual might have about a given herbal product or condition. Another possible strategy is to encourage physicians to ask their patients about the use of complementary and alternative medicine.

The market of herbal remedies and dietary supplements has to ensure: (a) security in composition, (b) definition of influence of metabolic aspects, including scientific validation and (c) regulatory aspects, e.g. the claims definition and relative influences.

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Table 1.

Species	Use-Toxicity	PA
BORAGINACEAE FAMILY		
Alkanna tinctoria aka *Lithospermum tinctorium* Alkannet	Skin diseases, diarrhea.	Triangularine
Amsinckia intermedia Fiddleneck	Horses and cows poisoned by it.	
Anchusa officinalisL. Alkannet, bugloss	Skin ulcers, expectorant and diuretic	
Borago officinalis L. Borage	Diuretic, inflammatory diseases and cough	Intermedine, lycopsamine.
Cynoglossum officinale hound's tongue, bugloss	diarrhea; skin bruises. Causes liver cancer	heliosupine, echinatine, lasiocarpine, cynoglossophine.
Heliotropium arborescens L. *H indicum L* . Da wei yao *H. popovii*, *H. lasiocarpum*; *H. eichwaldii*, *H. bacciferum* Heliotrope	*H. indicum:* Ulcer, wounds, local inflammation Livestock feed on heliotropium species -medicinal herb cause liver cancer; contaminated cereal crops cause human poisoning.	Heliotrine, indicine lasiocarpine
Lappula intermedia M.Popov Stickseed	-topically for sores and swellings. *L. intermedia* - Ascariasis, oxyuriasis, infantile malnutition	Intermedine
Lithospermum officinale Gromwell	Contraceptive, antipyretic, gout, kidney stones, diarrhea..	Lithosenine
Lithospermum erythrorhizon Zi cao .	skin diseases, antipyretic, antiphlogistic, produces tumors	Lithosenine, intermedine
Myosotis scorpioidesL. forget-me-not.	Sedative, tonic; externally eye wash.	myoscorpine, scropioidine, symphytine
Symphytum officinale L,(comfrey) *S. asperum Lepech*, *S. caucasicum Bieb.*, *S. peregrinum* *S. tuberosum*, *S. uplandicum* Nyman (Russian comfrey)	Causes liver damage in humans	intermedine, lycopsamine, symphytine, echimidine, symglandine.
Trichodesma africana also: *T. incanum*	Contaminated cereal crops cause human poisoning.	
ASTERACEA (COMPOSITAE) FAMILY		
Adenostyles alliariae Kern *Arnebia euchroma* (Ruan Zi Cao),	Lung disorders Causes HSOS	Lycopsamine, senecionine, seneciphylline, and spartioidine.

Chinese medicine Ageratum *conyzoides L.* Sheng hong ji	A. *conyzoides L.* Chinese medicine - antipyretic, common colds, malaria	
Brachyglottis repens	Crops are used for feeding animals. In horses, causes paralysis of the limbs.	Senecionine
Chromolaena odorata Fei ji cao *Crassocephalum crepidioides* Jia tong hao	Hemostatic; produces tumors *C. crepidioides* Cold, dysentery, urinary infection	Intermidine Jacobine
Emilia sonchifolia DC red tasselflower, Emilia's flower, Yang ti cao, Yi dian hong	Antipyretic influenza, cough, hemoptysis and bronchitis	senkirkine and doronine;
Eupatorium cannabinum L . Pei lan, *E. purpreum* - Joe Pye Weed *Eupatorium japonicum Thunb.* Hua zhe lan	E. cannabinum Influenza, cerebral stroke; *E. japonicum:* Measles, rheumatic pains and colds	supinine, rinderine, echinatine, intermidine and lycopsamine.
E. perforatum, boneset *E. rugosum* *E. cannabinum* hemp agrimony *E. fortunei* (Pai Lan)-Chinese medicine	E. purpureum fever; diuretic, lowering cholesterol; symptoms of summer-heat syndrome.	
Farfugium japonicum Kitam Lian peng cao	Colds and flu	Petasitenine, senkirkine
Gynura bicolor DC Guan yin xian,	Dysmenorrhea, tuberculous hymoptysis	Retrorsine
Gynura segetum Merr. Ju shan qi, Tu san chii	Hemoptysis, peripheral blood circulation disorder	Senecionine,seneciphylline
Ligularia hodgsonnii Hook *Lithospermum erythrorhizon* Zi Cao,	Anti-tussive traditional Chinese medicine produced in rural areas of Sichuan providence	otonecine-type PA, clivorine and ligularine,
Petasites hybridus PH Gaertn., B., Mey & Scherb. (Colts food), pestilence-wort or butterbur *P. spurius*. RCHB	Causes abdominal pain	senecionine, integerrimine, retrosine, seneciphylline, jacobine, senkirkine
Senecio argunensis Turcz. Yu yie qian li guang *S. aureus*- golden ragwort; *S. abrotarifolius,* *S.alpestre,* *S. bohemicus,* *S. bicolor* Tod. ssp. *cineraria,* *Senecio chrysanthemoides DC* Chien li kuang, *S. douglasii* *S. doronicum,* *S. fluviatilis* *S. fuchsii* *S. Illiciformis* *S. jacobaea L.,* tansy (European)	*S.argunensis*-Chinese medicine Antienteritis; *S. aureus* Diaphoretic and diuretic; high doses may induce abortion. *S. bicolor* has been used in eye drops to treat cataracts and conjunctivitis. *S. chrysanthemoides* Chinese medicine, Traumatic injury, breast abscesses *Senecio sp.*are used in Chinese medicine: febrile diseases, dysuria, inflammation, diarrhea, and, cataracts. Livestock - antispasmodic. *S. longilobus* - causes liver failure; *S. nemorensis* (Chinese medicine) Enteritis, hepatitis, boils	senecionine, riddelline, retrorsine, floridanine, monocrotaline, otosenine seneciphylline

ragwort *S. longilobus* *S. nemorensis L,* Huana wan S. *scandens* Qian Li Guang *S. sylvaticus,* *S. tomentosus* *S. vulgaris L.*	*Senecio* is used in African herbal medicine causes liver cancer. Contaminated cereal crops- human poisoning; causes infant death. Qianliguang is traditional herbal medicine growing in different locations of China. It is registered and sold as herbal remedy in western countries. It may lead to toxic adverse reactions. Quian li guang or Chiu li ming Oral and pharyngeal infection	
Tussilago farfara L. Coltsfoot Kuan Dong Hua (Chinese medicine).	lung disorders (chronic bronchitis, asthma), diarrhea	Senkirkine, senecionine
FABACAEA FAMILY		
Crotalaria sp. rattlebox *C. assamica Benth* Zi xiao rong (Nung gi li) Chinese remedy, *C. mucronata* Zhu zi tou Chinese medicine *C. juncea, C. nana, C. retusa, C. fulva; C. sessiliflora L*	Antipyretic and diuretic Contaminated cereal crops blamed for human poisoning.	crotananine, monocrotaline cronaburmin, retrorsine